Technical Note

Region of Interest (ROI) Scanning and Bleaching using Pulsed Lasers with the Olympus FluoView FV1000

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Introduction

Picosecond pulsed diode lasers are standard excitation sources for Fluorescence Lifetime Imaging (FLIM) and Fluorescence Lifetime Correlation Spectroscopy (FLCS) in conjunction with Laser Scanning Microscopes (LSMs) such as the Olympus FluoView FV1000. In addition, they proved to be also a very effective tool for selective photomanipulation of the specimen.

Confining the scanning or bleaching area to a defined Region-of-Interest (ROI), requires an exact timing of the PicoQuant pulsed diode laser. For this purpose the diode lasers can be externally gated (i.e. switched “on” and “off”) on a nanosecond time scale. This gating is also advantageous for standard FLIM or FLCS measurements, since the pulsed lasers will only excite the probe during data acquisition, avoiding photo damage in periods without data acquisition.

In order to integrate the gating option of the pulsed lasers into the Olympus FV1000 setup, special electronic modules were developed by Olympus and PicoQuant. Using this equipment, the fast gating can be precisely controlled by the Olympus LSM software laser management system.

Setup and working principle

The gating pattern is defined in the Olympus LSM software and send to the PicoQuant laser driver via two additional necessary modules. From Olympus the external laser control module FV10-LDIF (see Fig. 1) is needed, which is directly connected to the LSM controller. After software configuration, this module can be controlled via the Olympus LSM software (see Fig. 2). If just one pulsed laser with 375nm or 405nm is coupled into the SIM-port for bleaching or cutting, the FluoView must be equipped with a FV10-COL-405 output collimator.

The fastest switching time of the Olympus FV10-LDIF laser control module is, however, only in a range of 1 µs and therefore too slow for the required gating signal of the PicoQuant laser driver (PDL 800-D, PDL 808 or PDL 828). To overcome this problem a special Trigger Modul (TMF 100, fsee Fig. 1) was developed, which finally allows for gating times of less than 10 ns, i.e. in between two laser pulses.

The temporal control of the gating signal is defined using the Olympus LSM software and effectively switches the pulsed laser on and off. The laser

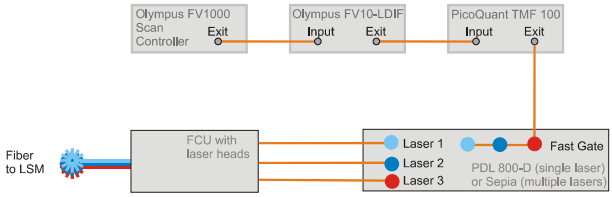

Fig. 1: Setup: Olympus LSM scan controller, Olympus control module FV10-LDIF, TMF100 “Trigger Module Fastgate”, PDL 800-D, 808 or 828 (“Sepia”) laser driver, laser heads in Laser Coupling Unit (LCU).

intensity during “on” times can, however, not be controlled by the software and is still done with the PicoQuant laser driver. The intensity bar in the Olympus software has therefore no effect.

The pulsed laser light can be connected to the FV1000 standard scanner (guiding the laser light into the scan head using a polarizing beamsplitter [1] or connected to the FV1000 SIM scanner. The SIM scanner allows for simultaneous imaging while the sample is bleached. Using the standard scanner, all pulsed wavelengths ranging from 375 nm up to 640 nm can be utilized.

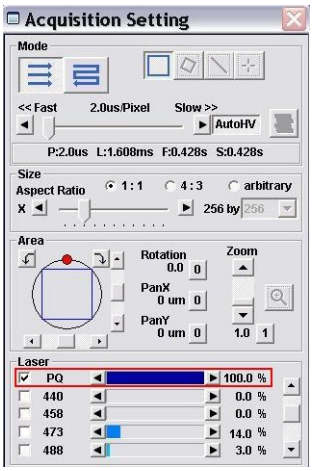

Fig. 2: After software configuration of the F10-LDIF the PicoQuant pulsed lasers are indicated (red rectangle) in the FV1000 software laser management and be manually en- or disabled. Laser power is controlled via the PicoQuant laser driver or Laser Coupling Unit (LCU).

Results

A typical application for the described setup is the selective photobleaching of ROIs in biological samples. Bleaching with picosecond pulsed lasers is more efficient due to the high peak power of the laser pulse in comparison to cw laser light. As can

be seen in Fig. 3, the ROI marked in image A is dark after the completion of the bleaching process (image B).

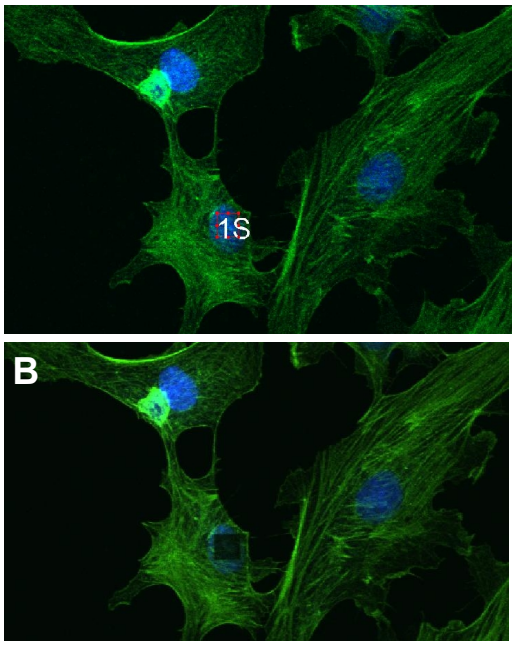

*Fig. 3: Bleaching of a ROI with a 405 nm pulsed laser, 45 µW laser power for 20 s using the standard scanner. **A**: Selection of the ROI (marked as 1S), **B**: Image after bleaching of the ROI of the FluoCells slide from Invitrogen, Carlsbad, CA, USA. The bleached region is completely dark.*

Pulsed laser light can also be used for cutting inside living cells as demonstrated by the group of Iva Tolic-Nørrelykke (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) [2], [3]. For laser ablation of the mitotic spindle 405 nm picosecond pulsed laser light of a LDH-P-C-405B laser head was coupled into the FV 1000 SIM scanner. The sample itself stained for visualisation which GFP was excited at 488 nm with a cw laser. The mitotic spindle in anaphase B was laser-irradiated in the center for 2 seconds applying a “tornado” scan (see Fig. Fehler: Referenz nicht gefunden). The spindle breaks into two segments that move towards each other while the astral microtubules gradually disassemble. The spindle fragments are finally positioned parallel to each other.

In conjunction with the PicoQuant FLIM and FCS upgrade, the described modules can also be used to control the switching of the pulsed lasers for FLIM, FLCS and FCS measurements to avoid photo damage in periods without data acquisition.

Conclusion

The described setup allows for external switching of PicoQuant pulsed diode lasers with the Olympus FluoView FV1000 laser scanning microscope. This enables selective illumination in user configurable ROIs which can for example be used for photobleaching, cutting, ablation, micro manipulation and uncaging.

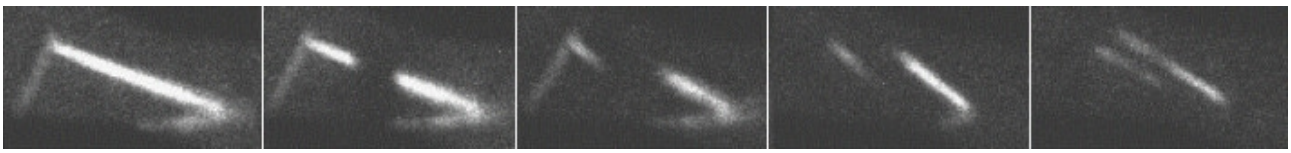

Fig. 4: Spindle cutting followed by unilateral elongation after 2 s irradiation with a 405 nm pulsed laser (LDH-P-C-405B) coupled into the SIM scanner of the FV1000. Data courtesy by Isabel Raabe, Sven K. Vogel, Jan Peychl, Iva Tolic-Nørrelykke, Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany

References

[1] Coupling of pulsed laser sources into the Olympus Fluoview FV300 / FV1000 using a polarization beam splitter, Krämer, B., Buschmann, V., Koberling, F., Technical note, PicoQuant GmbH, Germany

[2] Raabe,I.,Vogel,S.K., Peychl, J., Tolic-Nørrelykke, I., mentioned in Nature Volume 446, Number 7138, p. 937ff (http://www.nature.com/nature/journal/v446/n7138/pdf/446937a.pdf)

[3] Laser Cutting (Ablation) of the Yeast Mitotic Spindle using the LDH-P-C-405B, http://www.picoquant.com/products/ldh/ldh_ablation.htm

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